Higher Levels of Steroidogenic Acute Regulatory Protein and Type I 3β-Hydroxysteroid Dehydrogenase in the Scalp of Men with Androgenetic Alopecia

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TO THE EDITOR

Androgenetic alopecia (AGA) is the most common form of human baldness mediated by androgens. As most of the patients show normal levels of circulating androgens, a local *in situ* overproduction or/and androgen hyperresponse of the skin are suggested and supported by many studies, whereas higher levels of type I and type II 5α-reductase isozymes and androgen receptors have been demonstrated in frontal hair follicles than in occipital follicles (Hibberts *et al.*, 1998; Hoffmann, 2003). It remains unclear, however, whether the locally overproduced testosterone and 5α-dihydrotestosterone in the bald scalp derive from the cutaneous *de novo* androgen synthesis from serum cholesterol or from a shortcut, using serum dehydroepiandrosterone. Both are abundant, especially cholesterol. Four ''upstream'' enzymes including steroidogenic acute regulatory protein (StAR), cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc) and cytochrome P450 17α-hydroxylase/17,20-lyase (P450c17), and steroid 3β-hydroxysteroid dehydrogenase (3β-HSD) are responsible for the early steps of androgenesis from cholesterol to dehydroepiandrosterone. StAR regulates the first committed rate-limiting step by controlling the delivery of cholesterol from the outer membrane to the inner membrane of mitochondria, where cholesterol is further converted to pregnenolone by P450scc complex (Miller, 2002). Pregnenolone undergoes 17α-hydroxylation by microsomal P450c17 to form 17-hydroxypregnenolone and then is transformed to dehydroepiandrosterone by the 17,20-lyase activity of P450c17. Dehydroepiandrosterone is then converted by 3β-HSD to androstenedione, the substrate for testosterone.

We studied regional differences of the bald frontal–parietal *versus* the occipital scalp in the gene expression of the foregoing four enzymes in male AGA by using a quantitative, competitive RT-PCR method (Tsai and Wiltbank, 1996) (Figure 1a and b). Totally, 51 male AGA patients at stages III–VII according to the modified Norwood/Hamilton classification were recruited, with 25 patients (mean age 34.9 years, range 24–52 years) free of medication and 26 patients (mean age 37.3 years, range 21–58 years) taking 1 mg finasteride daily for at least 3 months. The study was conducted according to the Declaration of Helsinki Principles and approved by the Medical Ethical Committee of the Chang Gung Memorial Hospital, with written informed consent given by the participants. Paired data from both areas were obtained from 48, 46, and 20 of the total 51 patients for StAR, 3β-HSD, and P450scc, respectively. The estimated amount of the mRNA of each gene in the bald scalp, as normalized to the expression level of glyceraldehyde 3-phosphate dehydrogenase, was most abundant for StAR, followed by 3β-HSD, and much less for P450scc and P450c17. In 19 patients with detectable P450c17 mRNA, 10 had only expression in the bald scalp, whereas three only in the occipital scalp, and six had paired data from both areas. DNA sequencing of the PCR amplification of 3β-HSD (Tsai *et al.*, 2001) confirmed the gene expression of type I instead of type II 3β-HSD in the skin, even in the bald scalp (Dumont, 1992).

When the mRNA expression in the occipital (H) scalp was taken as 1, significantly higher levels of StAR and 3β-HSD were demonstrated in the bald parieto-frontal (B) scalp as compared to the occipital scalp, represented by elevated B/H ratio ($P=0.0117$ and 0.0164, respectively, paired *t*-test) (Figure 1c and d). No regional difference existed regarding P450scc ($P=0.3752$). The regional difference of the examined genes was not consistent among the examinee, that is, some AGA patients with overexpression of StAR in their bald scalps showed normal or lower levels of 3β-HSD in the same area, and *vice versa*. No association existed between the expressional intensity of StAR, 3β-HSD, or P450scc and the baldness severity (Pearson's product-moment correlation test) or between them and the patients' age (multiple linear regression). Correlation among the four examined genes with each other was insignificant (analysis of variance test). There was no difference between the finasteride treatment and non-treatment group regarding the regional expressional intensity (B/H) of each examined gene (paired *t*-test). All the statistics and analysis were performed using SAS PC software (version 9.1; SAS, Cary, NC).

Immunocytochemistry performed on cultured SZ95 sebocytes showed localization of StAR mainly in the perinuclear portion of the cytoplasma (Figure 2a). The smaller rather undiffer-

Abbreviations: AGA, androgenetic alopecia; 3β-HSD, steroid 3β-hydroxysteroid dehydrogenase; P450c17, cytochrome P450 17α-hydroxylase/17,20-lyase; P450scc, cytochrome P450 cholesterol side-chain cleavage enzyme; StAR, steroidogenic acute regulatory protein

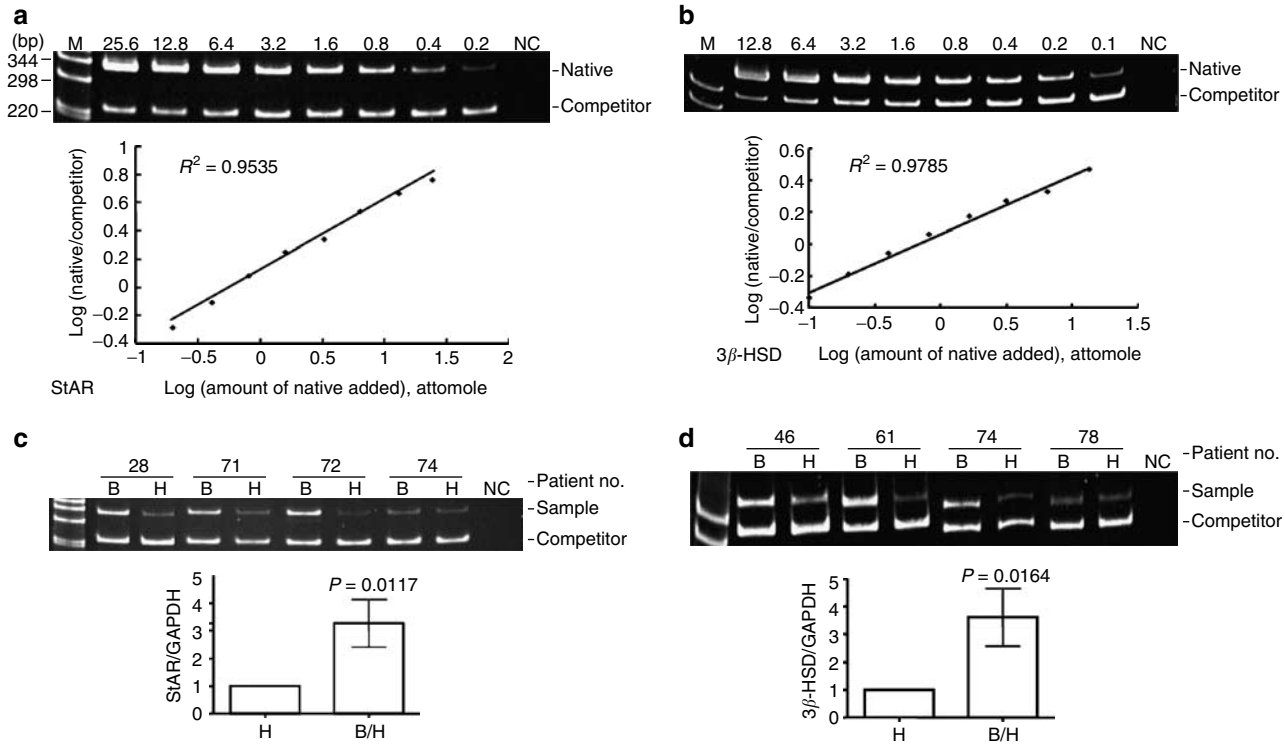

Figure 1. Detection and quantification of mRNA. After preparation and construction of the native and competitive plasmids for *in vitro* transcription of respective RNAs, the standard curves of quantitative competitive RT-PCR for each measured genes including (**a**) StAR and (**b**) type I 3β-HSD1 were generated by analyzing the intensity of bands derived from ethidium bromide-stained PCR products. A 2-fold serial dilution of native RNA was reverse transcribed and PCR amplified in the presence of 1 am competitor. The band intensity was quantified by AlphaImager computer software. Representative gel picture showing PCR products amplified from paired specimens taken from the bald fronto-parietal (B) and hairy occipital (H) scalps of men with AGA during hair transplantation. After calibration by the expressional intensity of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a B/H ratio for the measured mRNA levels was obtained from each individual subject regarding (**c**) StAR and (**d**) 3β-HSD1, respectively. Mean value of B/H ratio was calculated for StAR and 3β-HSD1 from 48 and 46 patients, respectively. As compared with the enzyme expression in the occipital scalp (H, taken as 1), significantly higher levels of StAR and 3β-HSD1 were found in the bald fronto-parietal scalp ($P<0.05$). NC, negative control by omitting reverse transcriptase during the RT procedure.

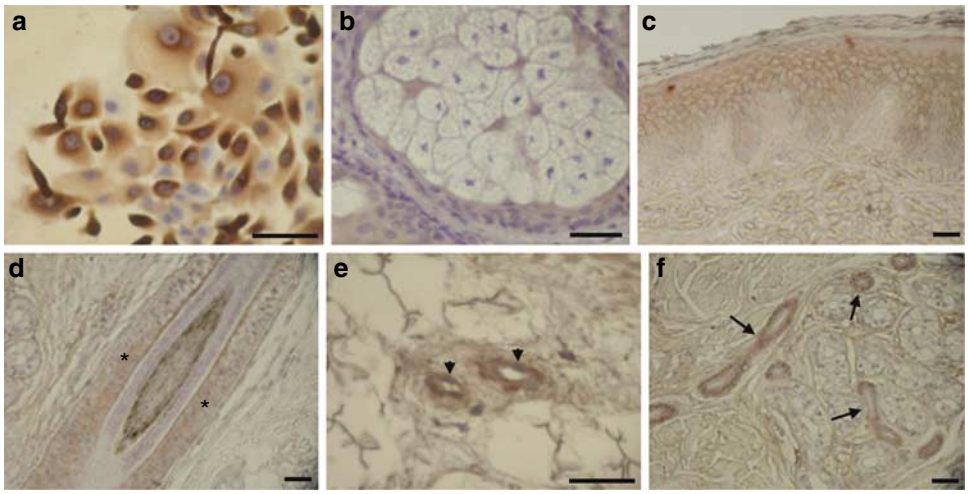

Figure 2. Immunodetection of StAR *in vitro* and *in vivo*. A specific rabbit polyclonal antibody raised against amino acids 1–285 representing full-length StAR of human origin (Santa Cruz, Santa Cruz, CA) was used and diluted to 1:100 and 1:50 in diluent (DAKO, Glostrup, Denmark) for cultured cells and for skin derived from vertical scalp of a 24-year-old man without AGA, respectively. StAR was detected mainly in (**a**) the perinuclear cytoplasm of the cultured SZ95 sebocytes, (**b**) basal layer of the sebaceous glands, (**c**) epidermal keratinocytes, (**d**) outer root sheath of hair follicles (asterisk), (**e**) vascular tissues (arrowhead), and (**f**) eccrine sweat ducts (arrow) but not eccrine sweat glands. Note the stronger expression of StAR protein in the small undifferentiated sebocytes (**a** and **b**). Bar = 20 μm.

entiated sebocytes displayed more strongly the StAR protein. Immunohistochemistry showed strong expression of StAR in the basal layer of sebaceous glands (Figure 2b), epidermal keratinocytes (Figure 2c), outer root sheath of hair follicles (Figure 2d) but not dermal papilla cells (data not shown), vascular tissues (Figure 2e), and eccrine sweat ducts but not eccrine sweat glands (Figure 2f). It is noteworthy that in hair follicles, androgen receptor expression was demonstrated to be restricted to dermal papilla cells, whereas in sebaceous glands, it was observed by some authors to predominate in the basal sebocytes but by others to exist both in basal cells and differentiating sebocytes (Thornton *et al.*, 2003; Pelletier and Ren, 2004). Our finding of a stronger expression of StAR protein in the undifferentiated sebocytes seems to be in line with the observation that androgens stimulate the proliferation of human sebocytes (Zouboulis *et al.*, 1999), contrary to the data from rat preputial sebocyte model, which postulated the major effect of androgens was on an early step in cell differentiation (Miyake *et al.*, 1994; Rosenfield *et al.*, 1999).

In steroidogenic cells, tropic hormones can regulate the StAR protein expression by cAMP-dependent mechanisms (Manna *et al.*, 2003). A recent study showed that prostaglandin E_2-stimulated StAR gene activation is mediated by E prostanoid receptor 2-coupled cAMP–protein kinase A pathway in peripheral endocrine tissues (Sun *et al.*, 2003). cAMP is also required for the growth and differentiation of rat preputial sebocytes (Rosenfield *et al.*, 2002). These data including our own seem to suggest the close interaction between androgenesis and prostaglandins in regulation of the sebocyte development (Zouboulis, 2005).

Upregulation of StAR and 3β-HSD in the bald scalp of AGA patients may simply reflect marked hyperplasia of sebaceous glands contrasted with the miniaturized hair follicles or imply the active *de novo* biosynthesis of androgens in pilosebaceous units. Given the juxtaposition of hair follicle and sebaceous glands, a kind of paracrine effect originating from sebaceous gland-secreted androgens on the growth and development of hair follicles is possible. Separate isolation of sebaceous glands and hair follicles to determine the difference of gene expression in bald and hairy scalp would help clarify the contributory role of each steroidogenic organ.

The significance of high StAR levels but limited gene expression of P450scc and P450c17 in the skin as well as in most other steroidogenic tissues is not clear (Slominski *et al.*, 1996; Thiboutot *et al.*, 2003). As StAR is an acutely regulated protein with very short half-life, its regulation at the transcriptional level may be required in order to provide sufficient mRNA transcripts for synthesizing protein that carries cholesterol to the inner mitochondria. On the other hand, the P450c17 expression in diseased state may be different, as P450c17 mRNA was more commonly detected in the bald than occipital areas of our AGA patients.

Enhanced expression of StAR and 3β-HSD in the bald area further implies that locally aberrant production of androgens may play a significant role in the development of the disease. Comparable data from normal controls without AGA including functional studies on the enzyme activities are needed to determine the role of *de novo* androgenesis in the pathogenesis of AGA.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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WenChieh Chen[1], Shaw-Jenq Tsai[2], Chen-Yu Liao[1], Ren-Yeu Tsai[3], Yi-Ju Chen[4], Bi-Jen Pan[5], Chiu-Ling Hung[1] and Christos C. Zouboulis[6]

[1]Department of Dermatology, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University, Kaohsiung, Taiwan; [2]Department of Physiology, College of Medicine, National Cheng Kung University, Tainan, Taiwan; [3]Skin Laser Center, Taipei Metropolitan Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan; [4]Department of Pathology, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University, Kaohsiung, Taiwan; [5]Faculty of Public Health, College of Health Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan and [6]Departments of Dermatology and Immunology, Dessau Medical Center, Dessau, Germany. E-mail: wchen@cgmh.org.tw

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The Killer Cell Immunoglobulin-Like Receptor Genes Do Not Confer Susceptibility to Psoriasis Vulgaris Independently in Chinese

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TO THE EDITOR

It has been suggested that psoriasis vulgaris (PV) is a multifactorial disease and that environmental factors may trigger PV in genetically susceptible individuals (Elder *et al.*, 2001; Lebwohl, 2003). At the *PSORS1* locus on chromosome 6p21.3, the human leukocyte antigen (*HLA*)-*Cw*0602* allele strongly associates to susceptibility to psoriasis in various populations (Mallon *et al.*, 1999; Zhang *et al.*, 2002). Aside from HLA-C, the implication of natural killer and natural killer-T (NK/NKT) cells in PV and psoriatic arthritis (PA) has also drawn much attention recently (Martin *et al.*, 2002; Holm *et al.*, 2005). NK/NKT cells were present in chronic psoriatic plaques and the NK/NKT cell line was shown to induce psoriasis in a severe combined immunodeficient mouse model (Nickoloff *et al.*, 2000). NK/NKT cell function is regulated by activating and inhibitory receptors present on the cell surface. Among them, the killer cell immunoglobulin-like receptor (KIR) is the major molecule recognizing HLA-C and is encoded by the KIR gene family clustered on chromosome 19q13.4. KIRs include activating KIRs such as KIR2DS and inhibitory KIRs such as KIR2DL. KIR2DS1 can recognize group 2 HLA-C including HLA-Cw2, -Cw4, -Cw5, -Cw6, and related alleles which carry lysine at amino acid 80 (Lys80). On the other hand, KIR2DS2 can recognize group 1 HLA-C such as HLA-Cw1, -Cw3, -Cw7, –Cw8, and related alleles which carry Asn80. *KIR2DS1*, *KIR2DS2*, and *KIR2DL5* have been associated with PA and PV (Luszczek *et al.*, 2004; Suzuki *et al.*, 2004; Williams *et al.*, 2005). Furthermore, subjects who are homozygous for *HLA-Cw* group 1 or 2 and positive for *KIR2DS1* and/or *KIR2DS2* are found to be most susceptible to developing PA (Nelson *et al.*, 2004), and patients with guttate psoriasis were associated with a distinct *HLA-C/KIR* genotype profile (Holm *et al.*, 2005).

In this study, we investigated the association of the KIR genes (*KIR2DS1*, *KIR2DS2*, *KIR2DL1*, *KIR2DL2*, and *KIR2DL5*) with PV in 178 Chinese patients with PV and 203 control subjects in Taiwan. All participants gave their written informed consent before enrolling in the study. The study was carried out with the approval of the institutional review board of the hospital and conducted according to the Declaration of Helsinki Principles. The 178 psoriasis patients included 125 males and 53 females with a mean age of 55.9 years (range, 7–87 years); the predominance of male PV patients in Chinese has also been reported in a previous study (Chen *et al.*, 2003). Ninety-two patients (51.7%) had an onset of psoriasis before the age of 40 years (early-onset psoriasis) and 86 patients (48.3%) had an onset at age 40 years or older (late-onset psoriasis). Sixteen (9%) of these patients reported a family history of PV. The control group comprised 203 healthy individuals (141 males and 62 females with a mean age of 53.9 years (range, 18–77 years)) and included voluntary blood donors, hospital staff, and medical students. Genotyping for *KIR2DS1*, *KIR2DS2*, *KIR2DL1*, *KIR2DL2*, and *KIR2DL5* was detected by PCR with sequence-specific primers (Gomez-Lozano and Vilches, 2002) with an internal positive control included in each PCR reaction. Genotyping for *HLA-Cw*0602* was also carried out using HLA Typing Kits (Lifecodes, Stamford, CT) and confirmed by sequence-based typing method (Chang *et al.*, 2004). The differences of the allele frequencies between the case and control subjects were assessed using χ^2 test or Fisher's exact test. Odds ratios, confidence intervals, and significance values were calculated using the Epi Info 2000 (CDC, Atlanta, GA).

As shown in Table 1, the genotype frequencies of activating *KIRs* (*KIR2DS1* and *KIR2DS2*) and inhibitory *KIRs* (*KIR2DL1*, *KIR2DL2*, and *KIR2DL5*) were similar between the PV patients and the controls. Therefore, *KIR2DS1*, a major susceptibility gene to PV found in the Caucasian and Japanese populations, was not a risk gene in the Chinese population; in fact, the *KIR2DS1* frequency in our PV patients was even

Abbreviations: HLA, human leukocyte antigen; KIR, killer cell immunoglobulin-like receptor; PA, psoriatic arthritis; PV, psoriasis vulgaris